

LATENT PROTEIN TREES

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Unbiased, label-free proteomics is becoming a powerful technique for measuring protein expression in almost any biological sample. The output of these measurements after preprocessing is a collection of features and their associated intensities for each sample. Subsets of features within the data are from the same peptide, subsets of peptides are from the same protein, and subsets of proteins are in the same biological pathways, therefore there is the potential for very complex and informative correlational structure inherent in these data. Recent attempts to utilize this data often focus on the identification of single features that are associated with a particular phenotype that is relevant to the experiment. However, to date there have been no published approaches that directly model what we know to be multiple different levels of correlation structure. Here we present a hierarchical Bayesian model which is specifically designed to model such correlation structure in unbiased, label-free proteomics. This model utilizes partial identification information from peptide sequencing and database lookup as well as the observed correlation in the data to appropriately compress features into latent proteins and to estimate their correlation structure. We demonstrate the effectiveness of the model using artificial/benchmark data and in the context of a series of proteomics measurements of blood plasma from a collection of volunteers who were infected with two different strains of viral influenza.

1. Introduction. Unbiased, label-free, mass spectrometry proteomics, sometimes called “shotgun” proteomics is a technique for measuring nearly all abundant proteins in a biological sample. Because of numerous technical advances it is becoming increasingly robust and sensitive, leading to greater effectiveness for the study of biological and medical questions (Aebersold and Mann, 2003; Service, 2008; Ping, 2009). While early work in this field met with a number of notorious failures (Petricoin et al., 2002; Baggerly et al., 2004; Zhang and Chan, 2005) due to overlapping peaks,

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batch effects and systematic noise, high accuracy spectrometers along with multiple fractionation techniques such as liquid chromatography and ion mobility have led to increased robustness as well as improved qualitative and quantitative results.

After summarization, data generated by this technology is typically presented as a $p \times n$ dimensional matrix of real valued *intensities* where the number of measured features p is typically orders of magnitude larger than n , as in microarray gene expression data. However, there are a number of important characteristics that distinguish mass spectrometry proteomics from gene expression data. First, each feature is a short peptide that has been enzymatically cut out of a parent protein, and parent proteins typically give rise to many such peptides. Second, only the more abundant of these features are typically identified (meaning that the peptide sequence and originating protein are known). Third, features that are present at lower abundances will typically have numerous missing values across the samples. Finally, while the error rate for assigning identifications to features is low, it is not zero, and this leads to some peptides with incorrect identifications.

Analysis approaches for these data can be performed at the feature level or at the protein level. The obvious consequence of performing analysis at the feature level is a significant loss of power due to the highly dependent nature of subsets of the features—particularly those that originate from the same protein. We prefer a dimension reduction approach in which groups of features are collected and summarized prior to analysis of associations between features and biological phenotypes. There are a number of approaches to this in the literature, almost all of which rely entirely on the identified features in the data set.

The simplest of these approaches involves direct summarization of all or some features that are identified for each protein either through averaging or robust summarization based on quantiles (Polpitiya et al., 2008). There are also a number of regression approaches which include fixed effects for protein, peptide and experimental group (Karpievitch et al., 2009), include an additional random effect for situations in which subjects are measured in replicate (Daly et al., 2008), or add additional interaction effects between treatment and features (Clough et al., 2009). These may assume constant or varying noise levels across isotope groups, and have been shown in some cases to exhibit better performance than naive summarization approaches that do not adjust for confounding factors (Clough et al., 2009).

We are aware of only one approach to the analysis of these data that examines correlation structure between data features (Lucas et al., 2012). This approach utilizes a latent factor model to aggregate features, and uses

priors on the loadings that are informed by identifications. This leads to aggregation of multiple features into “metaproteins”. This is a sparse factor modeling approach where non-zero loadings for factor i are biased toward features that are identified as originating from protein i . While this approach allows the utilization of unidentified features in the data, it fails to account for correlation structure that arises when multiple proteins are involved in the same pathways.

In this paper, we present an extension of [Lucas et al. \(2012\)](#) that explicitly models correlation structure between factors. We do this by incorporating a hierarchical structure on the latent metaproteins that allows borrowing strength between factors to estimate overall factor scores. We demonstrate improvements over both a generic sparse factor model [Carvalho et al. \(2008\)](#) and an earlier proteomics factor model [Lucas et al. \(2012\)](#), in terms of accuracy of factor estimates and eventual association with biological phenotypes. Finally, we demonstrate the incorporation of known correlation structure in the form of repeated measures in our analysis of a viral challenge data set in Section 6.

2. Motivating data. While the specifics of data generation may vary at different proteomics laboratories, the model we describe is appropriate for any high-accuracy mass spectrometry data. In general, the steps to data generation are as follows: (i) A biological sample is distilled to a solution containing those proteins that are of interest; (ii) The proteins in the sample are then broken up via trypsin; (iii) The processed sample is separated according to hydrophobicity using liquid chromatography. The time at which a particular constituent of the sample passes out of the chromatography column is called *retention time*; (iv) An electric charge is induced on the peptides; (v) The mass and intensity of these ions is measured in a mass analyzer. The intensity and ion masses are measured at a regular interval, called *sampling rate* and the resulting measurements form a trace with visible peaks, called *features*, that correspond to one or more peptides. Because the sampling rates are high relative to the size of these features, each feature spans a range of mass-to-charge ratios and retention times.

In nature, approximately 1% of all Carbon atoms are Carbon-13 (they contain an extra neutron). This leads to multiple features per peptide, each one containing a different, integer number of Carbon-13 atoms. These are collected into a single *isotope group* (IG) during preprocessing, and the intensity of this isotope group is estimated as the total volume under its associated features. In addition to multiple features from Carbon-13 substitution, a peptide may be present in the data set multiple times at different

charge states. These different charge states will have different mass to charge ratios and therefore result in multiple isotope groups per peptide.

There are inherently two different types of correlation present in label-free, unbiased proteomics data. First, each isotope group originates from a particular protein and there are typically many isotope groups per protein in the data set—particularly for proteins that are highly abundant and/or of large molecular weight in the original sample. Second, some collections of proteins are expected to behave similarly because they are part of the same biological pathways. This will result in correlation between proteins (and therefore correlation between isotope groups) that are of distinct etiology. In general, distinct sources of correlation are confounding without some additional information allowing us to distinguish them. In the case of proteomics, there are techniques for identifying the specific amino acid sequence of a subset of the isotope groups that are present at relatively high concentrations. These sequences are then associated to particular proteins through sequence alignment to proteins in a database (Nesvizhskii et al., 2003). We have then, for a limited subset of the isotope groups a (possibly imperfect) peptide sequence and originating protein, we call *annotation*.

The proteomics data we will be focused on was obtained from 43 patients as part of the DARPA H1N1/H3N2 viral challenge project (Zaas et al., 2009). From the entire pool, 24 patients were exposed to H1N1 and 17 were exposed to H3N2. For each patient, four samples were taken at different reference time points, baseline ($t = 0$), the time of maximum symptoms ($t = 1$) as well as $t = 0.2$ and $t = 0.8$. Each subject was labeled as symptomatic (SX) or asymptomatic (ASX) based on self-reported symptom scores, as well as viral culture. The samples of the H3N2 study were run in two batches with the initial pilot study containing only samples from time points $t = \{0, 1\}$ and the followup containing the $t = \{0.2, 0.8\}$ samples. In summary, we have $N = 172$ samples from two studies (H1N1 and H3N2) divided in three batches (H1N1, H3N2₁ and H3N2₂), two conditions (SX and ASX) and where the data itself is a matrix of approximately 40,000 IG expressions per sample. Peptide annotation was done using a combination of Mascot and PeptideProphet algorithms (Keller et al., 2002; Perkins et al., 1999). Isotope groups from the three batches were aligned using the algorithm described in Lucas et al. (2012). From all IGs, 13845 were successfully aligned across the H1N1 and H3N2 data sets. From the set of 4670 annotated IGs, only 1697 had annotations in both data sets. The set of annotations consists of 239 proteins from which only 106 are assigned to more than a single IG. The data has a relatively low overall missingness rate, most of them among low abundance IGs. However, missing values are unevenly distributed: H3N2₁

having 10.3% missingness, H3N2 0.7% and H1N1 up to 2.5%. Two samples were removed from subsequent analysis because they had more than 30% missing values in the set of annotated IGs.

3. Model definition. We model a sample n of batch m consisting of p IG expressions, \mathbf{x}_n^m , as an extended factor model separated into four effects, namely batch, systematic, protein expression and noise

$$(3.1) \quad \mathbf{x}_n^m = \boldsymbol{\mu}^m + \mathbf{A}\mathbf{z}_n + \mathbf{B}\mathbf{w}_n + \boldsymbol{\epsilon}_n,$$

where \mathbf{x}_n^m , $\boldsymbol{\mu}^m$, \mathbf{z}_n , \mathbf{w}_n and $\boldsymbol{\epsilon}_n$ are $p \times 1$ vectors. In particular, $\boldsymbol{\mu}^m$ is the mean expression vector of batch m , factors $\mathbf{z}_n = [z_{1n} \dots z_{N_F n}]^\top$ are meant to capture systematic N_F effects, \mathbf{w}_n is the expression level of N_P proteins for sample n , \mathbf{A} and \mathbf{B} are $p \times N_F$ and $p \times N_P$ loading matrices for the systematic effects and protein expressions, respectively, and $\boldsymbol{\epsilon}_n$ is measurement idiosyncratic noise. Systematic effects are included in the model for the sole purpose of cleaning the data as much as possible from batch effect specific and technical noise, with the aim to obtain protein profiles $\{\mathbf{w}_n\}$, that better reflect true biology rather than technical variability. Provided that protein expression is not directly observed and because profile vectors $[w_{k1} \dots w_{kN}]$ are likely to be estimated from IGs that belong to multiple proteins, from now on we refer them as *latent proteins*. A-priori, we let each IG to be associated only to a single latent protein, say k , meaning that row of \mathbf{B} contain just one non-zero entry.

Identifiability in the model of equation (3.1) follows from well known results for standard factor models and it should not be an issue for several reasons: (i) Confounding between systematic effects and metaproteins is very unlikely because \mathbf{A} is essentially dense and \mathbf{B} is actually a vector—assuming fixed IG-protein associations. (ii) \mathbf{w}_n does have a sign ambiguity that can be easily handled by letting \mathbf{B} have non-negative entries. (iii) \mathbf{z}_n is can be identified up to scale and permutations if N_F is small enough or for arbitrary N_F as long as its distribution is non-Gaussian (see Kagan, Linnik and Rao, 1973). Scale and permutation ambiguities are not of great concern here because we are not interested on the interpretation of systematic effects.

3.1. Prior specification. We need to specify prior distributions for each one of the elements in the right hand side of equation (3.1). Measurement noise is set to a zero-mean Gaussian with diagonal covariance matrix $\boldsymbol{\Psi}$, to allow for different noise variances for each IG. Entry specific priors for $\boldsymbol{\Psi}$ are set to flat inverse gamma distributions with shape $t_s = 1.1$ and rate $t_r = 0.001$, the former to keep the variance bounded away from zero. Mean

batch effects have Gaussian priors with mean $t_m = 8$ and small precision $t_p = 0.01$, set mainly based on the overall mean expression of the data.

3.1.1. Systematic effects. We define systematic effect as a portion of variability expressed in a large collection of isotope groups that cannot be classified neither as non-specific measurement noise nor biological variability, meaning that it is more likely due to technical variability. These effects are usually characterized by high levels of correlation across many isotope groups, but potentially only in a subset of the samples (for example, only those in one batch). We capture the first part through the use of independent Gaussian priors on the elements of \mathbf{A} , which allows systematic effects to span the entire set of isotope groups. Aiming to allow individual samples to be largely dropped from specific systematic factors, we utilize independent Laplace priors for the elements of \mathbf{z}_n . These are parameterized as scale mixtures of Gaussians with exponential mixing densities to facilitate inference (Henao and Winther, 2011). We consider that the number of systematic factors N_F is not critical because we are not concerned about the interpretability of matrix \mathbf{A} . Besides, we have observed empirically that the variance explained by the systematic effect factors saturates quickly as N_F increases. However, we decided to place an automatic relevance determination (ARD) prior on \mathbf{A} (Neal, 1996). In particular, being a_{ij} and z_{jn} elements of \mathbf{A} , and \mathbf{z}_n , respectively, we have

$$\begin{aligned} a_{ij} &\sim \mathcal{N}(0, \rho_j), & \rho_j &\sim \text{Gamma}(r_r, r_s), \\ z_{jn} &\sim \mathcal{N}(0, \tau_{jn}), & \tau_{jn} &\sim \text{Exponential}(\lambda^2), \quad \lambda^2 \sim \text{Gamma}(\ell_s, \ell_r), \end{aligned}$$

where ρ_j is a shared factor-wise variance for the columns of \mathbf{A} , τ_{jn} is an auxiliary variance with exponential mixing so marginally, $z_{jn} \sim \text{Laplace}(\lambda^2)$ (Andrews and Mallows, 1974). We further place a gamma hyperprior on the rate of the Laplace distribution with parameters $\ell_s = 4$ and $\ell_r = 2$. The mechanism behind an ARD prior is rather intuitive, for large values of ρ_j will correspond to small values of the j -th column of \mathbf{A} , thus virtually *switching off* the entire effect. Setting $r_r = 1.1$ and $r_s = 0.001$ will encourage the desired behavior. In practice, the *effective* number of factors can be determined by thresholding ρ_j or the elements of \mathbf{A} column-wise.

3.1.2. Latent protein profiles. We make two assumptions regarding isotope group expression. One is that each isotope group originates from only one latent protein and the other is that latent proteins may correlate with each other due to biological pathway activity. To model the first feature, we

set a prior hierarchy as follows

$$b_{i,u_i}|u_i \sim \mathcal{N}_+(0,1), \quad u_i|\mathbf{v}_i \sim \text{Discrete}(\mathbf{v}_i), \quad \mathbf{v}_i|\alpha \sim \text{Dirichlet}(\alpha \mathbf{1}_{N_P}),$$

where $b_{i,j} = 0$ if $j \neq u_i$, $\mathcal{N}_+(\cdot)$ is the Gaussian distribution truncated below zero and where the i -th IG is associated with the latent protein indexed by u_i with probability \mathbf{v}_i . This means that the vector \mathbf{u} serves as labeling variable for IGs. The conjugate prior for the vector of N_P probabilities \mathbf{v}_i , is set using a shared concentration parameter α . For the latter, we provide a flat gamma prior with parameters $a_s = 1$ and $a_r = 1$ (see [Escobar and West, 1995](#)).

We know that groups of proteins might have similar expression profiles for different reasons, for example because they are structurally similar, mediate similar biological processes, share a pathway, etc. In order to capture this structure, we place a prior over binary trees on the N_P latent proteins. This allows us to model correlation among metaproteins and leads to an interpretable representation of isotope groups, latent proteins and their interactions. Figure 1 illustrates the concept for a particular setting with $p = 15$ IGs distributed in $N_P = 5$ proteins. We can see a hierarchical clustering structure in which for instance latent proteins w_1 and w_2 are more similar than w_4 and w_5 , thus more correlated. The *pseudo time* t_j at which two nodes merge into v_j acts as similarity measure so that more alike latent proteins merge sooner in time, allowing us to directly quantify their pairwise or group-wise similarities. The proposed hierarchy is an implementation of the Kingman's coalescent ([Kingman, 1982a](#)), and reflects the idea that isotope groups and latent proteins lay in different levels and that protein pathways are proxies for the average profiles of collections of proteins.

Given a tree structure, $\{\mathbf{t}, \boldsymbol{\pi}\}$, where \mathbf{t} is the vector of merging times and $\boldsymbol{\pi}$ is the set of partitions at each level of the tree, we specify the relationship between node v_j and its parent node n_k (or w_k at the leaves) through a multivariate Gaussian transition probability and set the following prior hierarchy

$$(3.2) \quad \mathbf{v}_j|\mathbf{v}_k, t_j, t_k, \boldsymbol{\Phi} \sim \mathcal{N}(\mathbf{v}_k, (t_k - t_j)\boldsymbol{\Phi}), \quad \{\mathbf{t}, \boldsymbol{\pi}\} \sim \text{Coalescent}(N_P),$$

where \mathbf{v}_j is a N -dimensional row vector and $\boldsymbol{\Phi}$ is a covariance matrix encoding the correlation structure in \mathbf{v}_j . In a nutshell, a coalescent prior selects a pair to merge uniformly from partition π_j and sets merging times with rate 1, this is $t_k \sim \text{Exponential}(1)$. With no further constraints, this prior distribution leads to a uniform prior distribution over trees—the coalescent, that is independent of merging times and that is infinitely exchangeable ([Kingman, 1982a,b](#)). Different priors for $\boldsymbol{\Phi}$ add flexibility to the model, for

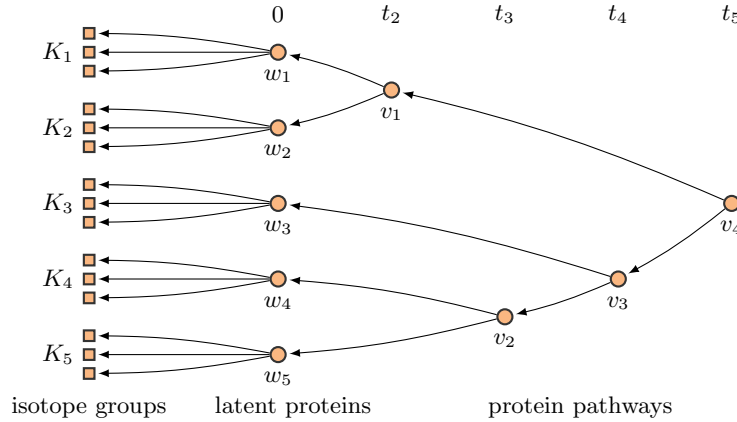


FIG 1. *Latent protein tree structure. Particular tree with $N_P = 5$ and three isotope groups assigned to each latent protein. The pseudo time variable t defines the merging points.*

example in the i.i.d. case, a diagonal Φ with independent inverse gamma prior distributions on each diagonal element will accommodate for differing levels of noise for different samples. In cases where there is reason to suspect for correlation across samples, a different prior could be used. In our analyses for instance we use inverse Wishart priors to model correlation due to sample replicates and Gaussian process priors for smoothness in time series data. Inference for hierarchy in equation (3.2) is carried out using an efficient sequential Monte Carlo Sampler introduced by Henao and Lucas (2012).

3.2. Inference. Model fitting is performed using Markov chain Monte Carlo (MCMC) to collect samples from the posterior of all parameters in the model, namely μ^m , \mathbf{A} , \mathbf{z}_n , \mathbf{B} , \mathbf{w}_n , Ψ , \mathbf{u} , π and Φ . The most relevant summaries involve posterior samples from the latent proteins, IG-protein assignments and the hierarchical structure encoded by the binary tree, π . Nearly all quantities of interest are updated using Gibbs sampling except for the tree components that require sequential Monte Carlo (SMC) sampling. In all the experiments we set the hyperparameters of the model to the values already mentioned unless otherwise stated. The upper bound for the number of factors is set to a conservatively large value, we have observed in practice that $N_F = \lfloor 2 \log(p) \rfloor$ is large enough. For tasks with p and N in the lower thousands and hundreds, respectively, we can expect the inference routine to take less than a couple of hours in a desktop machine. The entire sampling sequence is fully described in Appendix A.

Summaries for most of the important quantities of the model are computed in the usual way by means of histograms and empirical quantiles. Sum-

marizing trees on the other hand is not such an easy task because tree averaging is not a well defined operation. We could in principle use the pseudo time variable to build a pairwise distance matrix between latent proteins and then attempt to build a tree from a summary of such a *similarity* matrix. The problem being that we do not have any guarantee that this *average* of binary trees will produce binary tree as well. We tried this approach with both artificial and real data, and found that the tree built using means or medians of the similarity matrices collected during inference oftentimes produced trees with non-binary branching, thus not matching the prior assumption. In view of this, we decided to select a single tree from all the available samples using as criterion the marginal likelihood of the tree. This is a common practice in tree based models, see for instance Teh, Daume III and Roy (2008) and Adams, Ghahramani and Jordan (2010).

The source code and demo scripts for the model presented in this paper is written in MATLAB and C, and has been made publicly available at http://www.duke.edu/~rh137/lpt_v0.2.tar.

4. Artificial data. We begin with a set of experiments using artificially generated data in order to illustrate some of the features of our model and to perform some quantitative comparisons. We generated two datasets D_1 and D_2 of sizes $\{p, N, N_B, N_F, N_P\} = \{200, 80, 3, 5, 16\}$ and $\{400, 80, 3, 8, 32\}$, respectively. Denoting the elements of $\boldsymbol{\mu}^m$, \mathbf{A} , \mathbf{B} and $\boldsymbol{\Psi}$ as μ_i^m , a_{ij} , b_{ik} and ψ_i , respectively, we draw N observations of the model from the following hierarchy

$$\begin{aligned} \mathbf{x}_n^m &\sim \mathcal{N}(\boldsymbol{\mu}^m, \boldsymbol{\Sigma}), \\ \mu_i^m &\sim \mathcal{N}(8, 2), & m &\sim \text{Discrete}(N_B^{-1} \mathbf{1}_{N_B}), \\ a_{ij} &\sim \mathcal{N}(0, 0.1), \\ b_{i,u_i} &\sim \mathcal{N}_+(0, 1), & u_i &\sim \text{Discrete}(\mathbf{v}), \\ \psi_i^{-1} &\sim \text{Gamma}(1.1, 0.02), & \mathbf{v} &\sim \text{Dirichlet}(\boldsymbol{\alpha}), \\ \mathbf{S}^{-1} &\sim \text{Wishart}(\mathbf{I}, N_P), & \boldsymbol{\alpha} &\sim \text{Uniform}(0.8, 2.4), \end{aligned}$$

where $\boldsymbol{\Sigma} = \mathbf{A}\mathbf{A}^\top + \mathbf{B}\mathbf{S}\mathbf{B}^\top + \boldsymbol{\Psi}$, \mathbf{A} is a $p \times N_F$ matrix of systematic factor loadings, \mathbf{B} is a $p \times N_P$ matrix of latent protein loadings, \mathbf{S} is the covariance matrix of the latent protein profiles and $\boldsymbol{\Psi}$ is the noise diagonal covariance matrix, as in equation (3.1). We generated 50 replicates of each dataset and uniformly flagged 20% of its values as missing. We run our sampler for 2500 iterations, using the first 1500 as burn-in period. For this experiment, we set the distribution of the systematic factors to Gaussian, to match the

TABLE 1

Structural measures for artificial data. N_F is selected with threshold $\tau_j < 10^3$. Pairs in brackets are empirical 90% intervals across replicates. Best results in boldface letters.

Set	Method	N_F	Identity	Confusion
D_1	LPT	5 (4,7)	0.94 (0.88,1.00)	0.01 (0.00,0.04)
	sLPT	5 (4,7)	0.93 (0.81,1.00)	0.02 (0.00,0.06)
D_2	LPT	8 (7,10)	0.97 (0.93,1.00)	0.01 (0.00,0.02)
	sLPT	8 (5,10)	0.93 (0.86,0.97)	0.03 (0.01,0.06)

assumption made in Σ . Since we are not introducing correlation across samples, we set Φ to diagonal with independent gamma priors. The average number of systematic factors is selected with threshold $\rho_j < 10^3$. We label each latent protein by tabulating the IGs associated to it from vector \mathbf{u} and then picking the label having maximum count. We define *identity* as the percent of correctly labeled latent proteins, and *confusion* as the percent of variables incorrectly associated to their latent proteins. We compare our model (LPT) with (i) its simplified version without the tree structure inference we call sLPT, thus without covariance structure in the latent profiles (Lucas et al., 2012). Table 1 shows results for the structural components the model, namely number of systematic factors, identity and confusion. Results evidence our models ability to capture the associations between IGs and latent protein profiles through \mathbf{u} while properly handling systematic, batch effects and missingness in the data.

We can also asses the performance of our model in terms of covariance matrix and missing values estimation. We compare LPT and sLPT with a sparse factor model as proposed by (sFM, Carvalho et al., 2008) but equipped with the same priors for missing values and batch effects used by our model. For sFM we set the number of factors to $N_F + N_P = \{21, 24\}$, accordingly. In principle, the sparse model is flexible enough to estimate \mathbf{A} and \mathbf{B} but not \mathbf{S} for the model assumes independent profiles, similar to sLPT. Table 2 shows summaries of mean square error (MSE), mean absolute error (MAE) and maximum absolute bias (MAB) across replicates for the methods under consideration. As seen in Table 2, our model performs better than the other two alternatives.

The entire experiment was repeated for small variations in the hyperparameters of the models and the artificial data generator without considerable changes in the performance measures. In general terms, we observed good mixing in the sampler from exploratory and standard diagnostic tests. We also repeated the experiment with correlation across samples and inverse Wishart distribution for the matrix Φ with results similar to those shown in Tables 1 and 2.

TABLE 2

Performance measures for artificial data. *sLPT* is the simplified LPT and *sFM* is a sparse factor model. MSE, MAE and $10^{-1} \times \text{MAB}$ are mean squared error, mean absolute error and maximum absolute bias, respectively. Pairs in brackets are empirical 90% intervals. Best results shown in boldface letters.

Set	Measure	LPT	sLPT	sFM
Covariance				
D_1	MSE	1.462 (0.832,3.057)	7.297 (3.267,15.746)	9.184 (4.669,17.976)
	MAE	0.929 (0.702,1.378)	1.994 (1.401,2.884)	2.120 (1.475,3.223)
	MAB	0.606 (0.373,1.209)	1.065 (0.704,2.036)	1.558 (0.982,2.474)
D_2	MSE	1.242 (0.864,1.800)	4.141 (2.638,7.527)	4.314 (2.991,7.699)
	MAE	0.869 (0.725,1.067)	1.416 (1.174,1.857)	1.400 (1.137,1.849)
	MAB	0.658 (0.444,1.078)	1.079 (0.853,1.582)	1.396 (1.158,3.686)
Missing values				
D_1	MSE	0.212 (0.103,0.587)	0.253 (0.098,0.526)	1.604 (0.908,2.481)
	MAE	0.224 (0.199,0.298)	0.235 (0.193,0.281)	0.667 (0.531,0.892)
	MAB	0.776 (0.339,1.238)	0.722 (0.409,1.271)	0.956 (0.780,1.372)
D_2	MSE	0.225 (0.144,0.464)	0.260 (0.165,0.493)	1.716 (1.095,2.442)
	MAE	0.227 (0.209,0.255)	0.233 (0.214,0.267)	0.734 (0.601,0.910)
	MAB	0.900 (0.628,1.636)	0.929 (0.601,1.698)	1.027 (0.826,2.112)

5. Spike-in data. The benchmark dataset originally introduced by Mueller et al. (2007) consists of 6 samples measured in three replicates. Each sample is a mixture of six non-human purified proteins in different concentration levels spanning two orders of magnitude from 25 to 800 fmol. Figure 2(a) show in dashed lines ground truth concentrations in log-scale and scaled between 0 and 1. The raw data containing approximately 57000 IGs was filtered down to 1841 IGs after identification, annotation and exclusion of IGs with 50% missing values or with less than 10% of the maximum variance IG. We only count with annotations for 88 IGs, this is 4.7% of the set. In particular, the final dataset contains 18 observations and 1841 IGs labeled as 7 proteins, namely, ADH1-Y (12), ALDOA-R (20), CAH2-B (13), CYC-H (24), LYSC-C (9), MYG-H (10) and UKN (1753), with the number of IGs per protein in parentheses and UKN denoting unannotated IGs. The data matrix has a missingness of 30% more or less evenly distributed across observations. Since the dataset is relatively clean and all the samples were obtained in a single session, we do not expect systematic nor batch effects. However, we do expect high correlation due to replicates, thus we provide Φ with an inverse Wishart prior with $10 \times N$ degrees of freedom and scale matrix composed of 6 blocks of magnitude 0.9 and size 3 plus 0.1 times the identity matrix. Although, learning the degrees of freedom and the blocks/diagonal proportions will be more principled, we did not observe substantial changes in the results from small changes in the previously mentioned values. We run the

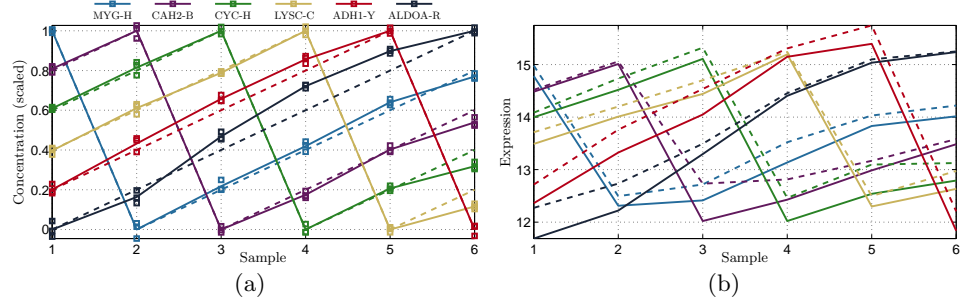


FIG 2. *Spike-in data profiles. (a) Ground truth (dashed) and estimated (solid) protein profiles scaled between 0 and 1. Replicates are shown as squares and solid lines are averages across replicates. (b) Median IG expression grouped according to the labeling obtained during inference and averaged across replicates. Dashed lines correspond to original data with missing values and solid lines to data with missing values replaced by their estimates. Credible intervals were omitted for clarity.*

sampler for 4000 iterations with a burn-in period of 2000.

Figure 2(a) shows the summary of the estimated latent protein profiles scaled between 0 and 1. Each circle represents a replicate, solid lines are averages across replicates and dashed lines represent the scaled ground truth (see Mueller et al., 2007). Summaries were computed using medians and credible intervals were omitted for clarity. Compared to the ground truth, our model does a pretty good job at capturing the underlying profiles of all 6 proteins of interest despite of the large amount of missing values and unannotated IGs.

Availability of the true protein profiles allows to quantitatively evaluate the performance of our model. We compare three different models in this case: (i) full i.i.d. latent proteins, meaning no tree structure prior; (ii) independent gamma distributions and diagonal Φ , assumes no correlation due to replicates and (iii) inverse Wishart prior for Φ with scale matrix as already described. Results of model (ii) also appear in Henao et al. (2012). Although the three models produce profiles similar to those shown in Figure 2(a), there are small differences. Table 3 indicates that in terms of MSE, MAE and MAB the summaries of the model with the inverse Wishart prior are more accurate compare to the other two that do not account for correlation across samples.

We can use the labeling vector \mathbf{u} to examine how unannotated isotope groups were labeled after inference. In particular, ADH1-Y went from having 12 IGs to 118, ALDOA-R from 20 to 307, CAH2-B from 13 to 240, CYC-H from 24 to 288, LYSC-C from 9 to 189 and MYG from 10 to 185. Figure 2(b)

TABLE 3

Performance measures for spike-in data. MSE, MAE and MAB are mean squared error, mean absolute error and maximum absolute bias, respectively.

Measure	no tree	tree with Φ prior	
		indep. gamma	inverse Wishart
$10^3 \times \text{MSE}$	2.524	1.899	1.661
$10^2 \times \text{MAE}$	3.172	2.983	2.494
$10^1 \times \text{MAB}$	1.443	1.252	1.213

shows median IG expression grouped according to the labeling vector \mathbf{u} and averaged across replicates to make easier comparisons against the ground truth in Figure 2(a). Dashed and solid lines correspond to data with and without missing values, respectively. For the latter, we have simply replaced the missing values with those estimated by our model. We see that for every protein, the inclusion of estimated missing values improves the expression average, mostly in the lower end of the expression range. We point out that a similar picture without using vector \mathbf{u} but the labeling from annotation instead, does not resemble at all the ground truth. This is because the original labeling comprises 88 IGs with a considerable amount of missing values.

6. H1N1/H3N2 viral challenge. We present now the case study based on the motivating data already described in Section 2. Here we will be using only the set of 4670 annotated IGs for which we have at least 2 IG per protein. The model has thus sizes $n = 172$, $N_B = 3$, $N_F = 16$ and $N_P = 106$. In this case, each observation can be seen as an element of a time series of length 4, $t = \{0, 0.2, 0.8, 1\}$. If we let latent proteins have Gaussian process priors with squared exponential covariance function and assuming no sample correlation across patients, we can compute the entries of Φ from

$$\phi(i, j) = c_{ij} \exp\left(-\frac{1}{\ell} d_{ij}^2\right) + \sigma^2 \delta_{ij},$$

where ℓ is the inverse length scale, σ^2 the idiosyncratic noise variance, $\delta_{ij} = 1$ only if $i = j$, $c_{ij} = 1$ only if i and j are from the same patient, and $d_{ij} = t_i - t_j$ is the time difference between pair $\{t_i, t_j\} \in \{0, 0.2, 0.8, 1\}$. Hyperparameters ℓ and σ^2 are updated using slice sampling (Neal, 2003). We run the inference procedure for 5000 burn-in iterations followed by 2000 samples to compute summaries. The whole procedure takes approximately 2.5 hours in a regular desktop machine with 4 cores. Mixing was monitored using both exploratory and standard diagnostic tests. Table 4 reports the resulting structural components of the model, namely previously described: number of systematic factors, N_F , identity and confusion. Besides, we define

TABLE 4
Structural measures for viral challenge data. N_F is selected with threshold $\tau_j < 10^3$ and stability with threshold 0.6.

N_F	Identity	Confusion	Stability	Unique
3	0.774	0.511	0.958	0.783

stability as the proportion of IGs having a single value in the label vector \mathbf{u} for at least 60% of the MCMC samples after the burn-in period. We also define *unique* as the proportion of latent proteins with distinct labels.

From Table 4 we see that nearly half of the IGs ended up with a protein label different to annotation. In real data this is not so surprising as the chances of miss-annotation considerably increase due to systematic effects, post-translational modifications, measurement error and alignment induced miss-labeling. As an example, consider the problem of aligning batches H1N1, N3N2₁ and N3N2₂. Initially, the three batches have different sets of notations that need to be matched to conform a common annotation set. We use the alignment algorithm described in (Lucas et al., 2012). From the 4670 IGs included in the model, only 36% of them shared annotation, whereas for the remaining 64% IGs, annotation was transferred from one of the batches to the other two. This means that more than half of the IGs are more prone to miss-annotation due to alignment. From the set of 2220 IGs (47%) that kept the label from annotation nearly half of them are part of the set of IGs with H1N1/H3N2 shared annotation, suggesting that IGs annotated simultaneously in all sets tend to be more reliable than those labeled by label transfer. The identity of the model on the other hand, indicates that 82 latent proteins match annotation when labeled by consensus of their IG members. Having 82 (78%) unique latent proteins implies that some proteins exist in multiple instances. For example, we count with 6 versions of APOB-H, which is in fact the most frequent protein in the set. Figure 3(a) shows the composition of all latent proteins. For each latent protein (column), we tabulate and sort the labels of its IG members. Darker colors represent proportions closer to 1. The first row is used to compute the consensus to determine identity. The red bar indicates whether the most frequent IG in a given latent protein is represented by less than 30% of the IGs assigned to it. The top bar shows in dark the 82 identified proteins. All in all, we see that for most latent proteins, the most frequent IG has an important contribution and that no latent protein has IGs from more than 17 different labels. Besides, we see correlation between the identified proteins and the proportion of their most frequent IG.

We can also use latent proteins as predictors of the symptomatic vs.

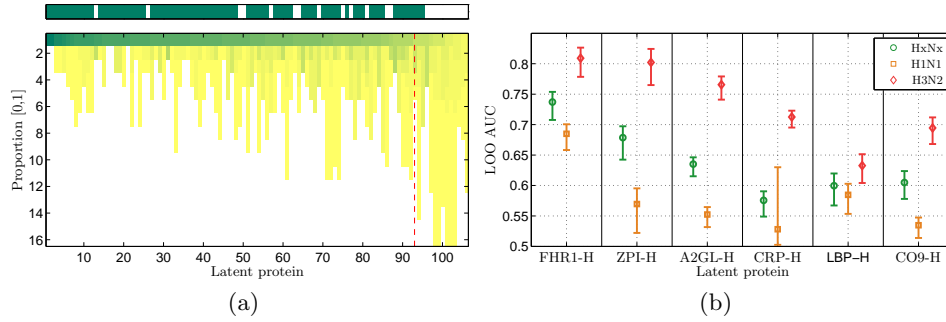


FIG 3. *Protein identification and status classification. (a) Latent protein sorted compositions, darker colors indicate proportions closer to 1. The red bar separates latent proteins in which the leading IG has proportion less than 30%. The top bar shows in dark the identified proteins. (b) Classification accuracy presented as AUC values estimated using leave-one-out cross-validation. Markers indicate median values and error bars cover 90% credible intervals.*

asymptomatic status of each observation in the dataset. For this purpose, we fit individual linear discriminant classifiers for each latent protein at each MCMC draw and estimate the classification accuracy as the area under the ROC curve (AUC, Receiver Operating Characteristic, [Fawcett, 2006](#)). Figure 3(b) shows results for six of the most discriminant latent proteins: FHR1-H, ZPI-H, CRP-H, LBP-H, A2GL-H and CO9-H, it shows in particular that FHR1-H has an overall decent performance. In addition, when treating H1N1 and H3N2 as separate classification tasks, we observe that H3N2 is somehow easier to classify as can also be seen quite clearly from Figure 3(b).

As described in Section 3, the prior distribution for the set of latent proteins allows us to build a binary tree representation of its elements in a hierarchical clustering fashion. When examining the resulting structure (see [Supplement A](#)) we found some straightforward groupings in the tree mostly corresponding to protein variants like APOC2-H and APOC3-H, CO8A-H, CO8B-H and CO8G-H, FIBG-H and FIBB-H, F13A-H and F13B-H, etc, all of them having similar profiles when looking at their estimated signatures (results not shown). In other cases, for instance CO4(a,b)-H and APOB-H, showing great diversity in their profiles and as a result rather spread in the structure.

Figure 4 shows the subtree corresponding to 4 of the discriminant proteins from Figure 3(b) along with a scatter of the expression values of each latent protein. Each panel in the figure shows expression in the y -axis and data grouping in the x -axis. Data to the left hand side of the dashed vertical line corresponds to the asymptotic set whereas the other side contain symptomatic observations. Each side is further grouped according to time, so points closer to the dashed vertical line are for $t = 0$ (green), then $t = 0.2$

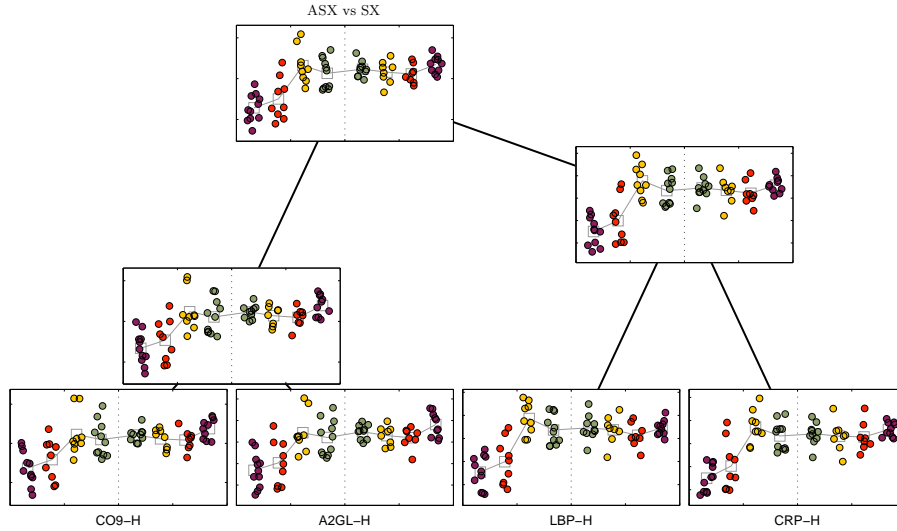


FIG 4. *Discriminant subtree. Each node is represented as a scatter of the samples from study H3N2 only. The dotted line separates samples labeled asymptomatic (left) and symptomatic (right). The x-axis groups samples according to time: red for $t = 0$, yellow for $t = 0.2$, red for $t = 0.8$ and purple for $t = 1$. The y-axis is the estimated latent/protein pathway expression. Solid lines connect group means. Note that time points $t = 0.8$ and $t = 1$ are nicely separated.*

(yellow), $t = 0.8$ (red) and the farthest to $t = 1$ (purple). The good separation of observations from times $t = \{0.8, 1\}$ is the main responsible for the classification results shown in Figure 3(b)

It should be noted that the DARPA study collected samples from multiple other sources, and that there is published, publicly available gene expression data from the peripheral blood of the same patients we have examined here. That data is analyzed in [Zaas et al. \(2009\)](#) and a time course trajectory model is developed on a more complete version of the data in [Chen et al. \(2011\)](#). Together with the proteomics data included in the supplementary material of this paper (see [Supplement B](#)), these offer interesting possibilities for future work into jointly modeling proteomics and gene expression data. We have briefly examined correlation between protein and matched gene expression in these data sets, but find that it is generally quite low. However, a brief examination of the top genes discovered in [Zaas et al. \(2009\)](#) and the five discriminative proteins elucidated here shows a high overlap in associated pathways. We suspect that these results, and in general joint analysis of these data is complicated by the tissue of origin. Specifically, it is not clear that the proteins in blood plasma originate from peripheral blood mononu-

clear cells (from which there is published gene expression data). Instead, it is likely that much of the observed protein expression is due to activities in organs such as the liver or kidneys and from the endothelial lining of blood vessels.

7. Concluding remarks. We have presented a factor model specifically designed for proteomics data analysis. It successfully handles broad scale variability that is known to come from technical sources (such as batch effects and isotope group specific noise) hence enabling us to estimate latent protein profiles that better describe biological variability. Our hierarchical representation of isotope groups, latent proteins and protein pathways provide us with detailed annotation uncertainty assessment, detection of possibly inaccurately annotated isotope groups and post-translationally modified proteins and clustering of proteins with similar expression profiles that reflect biologically related interactions. We have also shown that features of our model can be used to define predictive models based either on latent proteins or groups of latent proteins.

APPENDIX A: MCMC INFERENCE DETAILS

We describe next the MCMC analysis mostly based on Gibbs sampling. We provide then the relevant conditional posteriors and SMC based update details for the tree structure. To simplify notation, we use the following shorthands. Let $\mathbf{X}^m = [x_1^m \cdots x_{N_m}^m]$ and $\mathbf{X} = [\mathbf{X}^1 \cdots \mathbf{X}^{N_B}]$, where N_B is the number of batches, N_m is the number of samples in batch m and $N = \sum_{m=1}^{N_B} N_m$. Define $\mathbf{1}_k$ to be a k -dimensional row vector of ones and let $\tilde{\mathbf{X}}$ be the full data set with the appropriate means subtracted off, this is $\tilde{\mathbf{X}} = [\mathbf{X}^1 - \boldsymbol{\mu}^1 \mathbf{1}_{N_1} \cdots \mathbf{X}^{N_B} - \boldsymbol{\mu}^{N_B} \mathbf{1}_{N_{N_B}}]$, and $\mathbf{Z} = [\mathbf{z}_1 \cdots \mathbf{z}_N]$ and $\mathbf{W} = [\mathbf{w}_1 \cdots \mathbf{w}_N]$, systematic factors and latent protein matrices of sizes $N_F \times N$ and $N_P \times N$, respectively. For any matrix \mathbf{M} , define $\mathbf{M}_{i:}$ as its i -th row and $\mathbf{M}_{:,j}$ to be its j -th column.

Noise variance. Sample each element of the diagonal of $\boldsymbol{\Psi}$ from

$$\psi_i^{-1} | t_s, t_r \sim \text{Gamma} \left(t_s + \frac{N}{2}, t_r + c \right),$$

where t_s and t_r are respectively prior shape and rate and

$$c = \frac{1}{2} (\tilde{\mathbf{X}}_{i:} - \mathbf{A}_{i:} \mathbf{Z} - \mathbf{B}_{i:} \mathbf{W}) (\tilde{\mathbf{X}}_{i:} - \mathbf{A}_{i:} \mathbf{Z} - \mathbf{B}_{i:} \mathbf{W})^\top.$$

Batch means. Sample mean vector for batch m from

$$\boldsymbol{\mu}^m | t_m, t_p \sim \mathcal{N} \left(\mathbf{C} \left(t_m t_p + \boldsymbol{\Psi}^{-1} \sum_{n=1}^{N_m} \mathbf{x}_n^m - \mathbf{A} \mathbf{z}_n - \mathbf{B} \mathbf{w}_n \right), \mathbf{C} \right),$$

where $\mathbf{C} = (t_p + N_m \boldsymbol{\Psi}^{-1})^{-1}$, t_m and t_p are prior mean and precision, respectively.

Systematic effect factors. The conditional posterior of \mathbf{Z} , using a scale mixtures of Gaussians representation, can be computed independently for each element of the matrix using

$$z_{jn} | \tau_{jn} \sim \mathcal{N} \left(c_{jn} \mathbf{A}_{:j}^\top \boldsymbol{\Psi}^{-1} \boldsymbol{\epsilon}_{\setminus jn}, c_{jn} \right),$$

where $c_{jn} = (\mathbf{A}_{:j}^\top \boldsymbol{\Psi}^{-1} \mathbf{A}_{:j} + \tau_{jn}^{-1})^{-1}$ and $\boldsymbol{\epsilon}_{\setminus jn} = \mathbf{x}_n - \mathbf{A} \mathbf{z}_n - \mathbf{B} \mathbf{w}_n - \boldsymbol{\mu}^m | z_{jn} = 0$. The mixing variances τ_{jn} are exponentially distributed with rate λ^2 , hence the resulting conditional posterior is

$$\tau_{jn}^{-1} | \lambda^2 \sim \text{IG} \left(\sqrt{\frac{\lambda^2}{z_{jn}}}, \lambda^2 \right), \quad \lambda^2 | \ell_s, \ell_r \sim \text{Gamma} \left(\ell_s + \frac{1}{2}, \ell_r + \frac{1}{2} \sum_{j,n} \tau_{jn} \right),$$

where ℓ_s and ℓ_r are shape and rate priors, respectively. $\text{IG}(\cdot | \mu, \lambda)$ is the inverse Gaussian distribution with mean μ and scale λ (Chhikara and Folks, 1989). Each element a_{ij} from the loading matrix \mathbf{A} is sampled from

$$a_{ij} \sim \mathcal{N} \left(c_{ij} \boldsymbol{\epsilon}_{\setminus ij} \mathbf{Z}_{l:}^\top, c_{ij} \psi_i \right),$$

where $c_{ij} = (\mathbf{Z}_{j:} \mathbf{Z}_{j:}^\top + \psi_i \rho_j)^{-1}$ and $\boldsymbol{\epsilon}_{\setminus ij} = \tilde{\mathbf{X}}_{i:} - \mathbf{A}_{i:} \mathbf{Z} - \mathbf{B}_{i:} \mathbf{W} | a_{ij} = 0$. Then, column-wise precisions for \mathbf{A} are drawn from

$$\rho_j | r_s, r_r \sim \text{Gamma} \left(r_s + \frac{p}{2}, r_r + \sum_i a_{ij}^2 \right),$$

where r_s and r_r are prior shape and rate, respectively.

Protein profiles. The conditional posterior for latent proteins \mathbf{W} can be updated from

$$\mathbf{W}_{k:} | \mathbf{v}_k \sim \mathcal{N} \left(\mathbf{C} \mathbf{B}_{:k}^\top \boldsymbol{\Psi}^{-1} (\tilde{\mathbf{X}} - \mathbf{A} \mathbf{Z}) + \mathbf{C} \mathbf{S}_k^{-1} \mathbf{m}_k, \mathbf{C} \right),$$

where $\mathbf{C} = (\mathbf{B}_{:k}^\top \boldsymbol{\Psi}^{-1} \mathbf{B}_{:k} + \mathbf{S}_k^{-1})^{-1}$, with \mathbf{m}_k and \mathbf{S}_k being mean and covariance of the parent profile \mathbf{v}_k of $\mathbf{W}_{k:}$. Note that $b_{ik} = 0$ for all isotope groups not assumed to be part of this protein, and that these will not contribute to the update distribution for $\mathbf{W}_{k:}$. Besides,

$$b_{ik} | b_{ik} \neq 0 \sim \mathcal{N}_+ \left(c(\tilde{\mathbf{X}}_{i:} - \mathbf{A}_{i:} \mathbf{Z}) \mathbf{W}_{k:}^\top, c \psi_i \right),$$

where $c = (\mathbf{W}_{k:} \mathbf{W}_{k:}^\top + \psi_i)^{-1}$ and $\mathcal{N}_+(\cdot)$ is the Gaussian distribution truncated below zero. Now we can sample IG-latent protein assignments from

$$u_i | \alpha, \boldsymbol{\kappa}, t_s, t_r \sim \text{Discrete}(\mathbf{v}_i),$$

$$v_k \propto (\alpha + n_k) c^{-\frac{1}{2}} \left(t_r + \frac{1}{2} c^{-1} \tilde{\mathbf{X}}_{i:} \mathbf{W}_{k:}^\top \mathbf{W}_{k:} \tilde{\mathbf{X}}_{i:}^\top \right)^{-(t_s + \frac{N}{2})},$$

where n_k is the number of non-zero entries in column k of \mathbf{B} , $c = \mathbf{W}_{k:} \mathbf{W}_{k:}^\top$ and v_k is the k -th element of \mathbf{v}_i .

Protein pathway expression and tree structure. We sample the tree structure components \mathbf{t} , $\boldsymbol{\pi}$ and $\boldsymbol{\Phi}$, and the means and covariances of each internal node of the tree, \mathbf{m}_k and \mathbf{S}_k , respectively, using the SMC sampler described in Henao and Lucas (2012). In particular, $\{\mathbf{t}, \boldsymbol{\pi}\}$ are obtained for a number M of particles, as a leaves to root SMC pass, together with partial updates of the node parameters $\{\mathbf{m}_k, \mathbf{S}_k\}$. Next we use particle’s weights to sample a single configuration. The procedure is completed by resampling the hyperparameters of the covariance function and by completing the updates of the node parameters using the selected configuration, the latter as a root to leaves pass.

Missing values. For each missing value x_{in}^m corresponding to isotope group i , sample n and batch m , we simply use independent standardized Gaussian prior distributions.

Initialization. We start the model from maximum likelihood estimates of the less critical quantities, this is batch means $\{\boldsymbol{\mu}^m\}_{m=1}^{N_B}$ and noise variances $\boldsymbol{\Psi}$. Systematic factors \mathbf{Z} and latent proteins \mathbf{W} are initialized using standardized Gaussian distributions. The loading matrices \mathbf{A} and \mathbf{B} (non-zero elements only) were set to ordinary least squares estimates based upon already set \mathbf{Z} and \mathbf{W} , respectively. The vector of associations \mathbf{u} was set with the information obtained from annotation about IG-protein assignments.

SUPPLEMENTARY MATERIAL

Supplement A: Tree structure (<http://lib.stat.cmu.edu/aoas/?>). Figure showing the resulting tree structure for the H1N1/H3N2 viral challenge data.

Supplement B: Data (<http://lib.stat.cmu.edu/aoas/?>). H1N1/H3N2 viral challenge raw data

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